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Evaluation of platelet and its indices as a marker of neonatal sepsis: a prospective case control study

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ABSTRACT

Background: Neonatal sepsis is major cause of neonatal morbidity and mortality worldwide. Blood culture and sepsis screening are currently used method, but their utility is limited due to delayed reporting and increased cost. Platelet indices are one such set of parameters which can be helpful in the future diagnosis of neonatal sepsis. This study was aimed to evaluate the significance of platelet indices either alone or in combination with existing sepsis screen as a marker of neonatal sepsis.

Methods: Neonates admitted in the neonatal unit of Hospital and showing signs and symptoms of sepsis, and/or born to mothers with risk factor for sepsis were included in this study. Investigations sent for all these neonates included blood culture, sepsis screen (CRP, micro ESR, TLC, ANC, IT ratio) and platelet indices (Platelet count, MPV, PDW). **Results:** In present study, 81.12% neonates in case group had platelet count less than 1.5lacs/mm3 while in control group 20.91% neonates only had the same. This difference was statistically significant, (p<0.0001). Similarly, 70.91% neonates in case group had MPV more than >10.8 fl whereas in control group only 26.53% neonates had the same, with difference was statistically significant, (p<0.0001) Similarly, 65.81% neonates in case group and 34.69% in control group had PDW more than 19.1fl and this difference was statistically significant, (p=0.0001).

Conclusions: High PDW, high MPV and low platelet count are more associated with neonatal sepsis. So, platelet and its indices may be used as a sensitive marker to identify septic babies and it may be combined with existing sepsis screen to specifically exclude non-septic case.

Keywords: Mean platelets volume (MPW), Neonatal sepsis, platelets, Platelet distribution width (PDW)

INTRODUCTION

Neonatal sepsis is major cause of neonatal morbidity and mortality worldwide contributing around 38% of all deaths in neonates. Situation is even more worsened in low income underdeveloped countries. As per the National Neonatal Perinatal Database 2002-2003, the incidence of neonatal sepsis is 30 per 1000 live births. Neonates are fragile and can deteriorate rapidly, so rapid diagnosis and prompt management is required. Blood

culture and sepsis screening are currently used method, but their utility is limited due to delayed reporting and increased cost.² Due to these limitations we need to find parameters which will increase the sensitivity and specificity.

Platelet indices can be helpful in the future diagnosis of neonatal sepsis. Despite being a promising and convenient marker for sepsis, there have been only a few studies on the utility of platelet markers in neonatal sepsis. There are not many studies on this topic from our region. None of the available studies compares platelet indices with the existing sepsis screen in prediction of neonatal septicemia. So, looking at the paucity of studies in this issue, the present study is an effort to fulfill the gap in the existing literature and would evaluate the significance of platelet indices either alone or in combination with existing sepsis screen as a marker of neonatal sepsis.

METHODS

It was a Prospective Cohort (case control) study conducted in the neonatal division of Department of Paediatrics in collaboration with the Department of Haematology and Microbiology of Dr. S. N. Medical collage Jodhpur. Over a period of one year extending from January 2017 to December 2017. Study was Approved by Institute's ethical committee. Informed and written consent obtained from the parents of all enrolled neonates

This study was aimed to study the relation between neonatal sepsis and platelets and its indices (Platelet count, MPV, PDW). Primary objective was to evaluate platelet and its indices (platelet count, PDW, MPV) as a marker of neonatal sepsis, secondary objective was to determine if there is difference in platelet indices among-

- Term and pre-term,
- Early versus late onset sepsis

Neonates admitted in the neonatal unit of Hospital and showing signs and symptoms of sepsis, and/or born to mothers with risk factor for sepsis were included in this study. All neonates with culture positive sepsis, screen positive or clinical sepsis as per the CDC definition were defined as cases. Weight and gestational age matched healthy neonates served as control. Neonates having congenital and acquired cause of thrombocytopenia and platelet indices other than sepsis. i.e. Autoimmune disorder of platelets, allo-immune disorder of platelets and perinatal asphyxia were excluded from the study.

The sensitivity of sepsis screen for diagnosis of neonatal sepsis was found to be 93% in the study by Philips.³ Assuming a 0.05 one sided significance level, 80% power and a sensitivity of at least 85% with the new test (platelet indices), we enrolled 196 cases and an equal number of controls. Investigations sent for all these neonates included blood culture, sepsis screen (CRP, micro ESR, TLC, ANC, IT ratio) and platelet indices (Platelet count, MPV, PDW). For these tests we drew approximately 2 ml of venous blood from each neonate through peripheral veins.

On the basis of reports of the blood culture and sepsis screen, the neonates were classified into culture positive sepsis, screen positive, clinical sepsis with blood culture negative and no sepsis on the basis of definitions

provided by CDC. The clinical details of all cases and controls and their lab investigation results were recorded in the case record form. The sensitivity, specificity of platelet indices for diagnosis of neonatal sepsis was calculated. Among the cases with a positive blood culture analysis was done to see if there is a difference in platelet indices between Gram positive and Gram-negative sepsis.

In present study, p value < 0.05 was considered as significant with either negative or positive correlation on account of biological variability. Frequency along with percentage was provided for age groups, sex, weight and gestational age by two study groups that is, case and control. Differences in the distribution of these variables between case and control groups were tested through Chisquare test.

Differences in mean values of platelet count, MPV and PDW were assessed by t-test for independent groups. Between group differences of cut off groups of platelet indices were assessed using Chi-square test again for sex, weight, and gestational age and blood culture.

Seven combinations for marker of sepsis were created based on platelet count, MPV and PDW cut offs and compared with the gold standard method to measure sepsis. These combinations are,

- Platelet count <1.5 lacs/mm³,
- MPV >10.8 fl,
- PDW >19.1fl,
- Platelet count <1.5 lacs/mm³ and MPV >10.8 fl and PDW >19.1fl.
- Platelet count <1.5 lacs/mm3 and MPV >10.8fl,
- Platelet count <1.5 lacs/mm3and PDW >19.1fl,
- MPV >10.8fl and PDW > 19.1fl.

RESULTS

The study groups were homogenous with respect to demographic profile in term of birth weight (p=0.755), age of onset (p=0.409) and gestation age (p=0.409) and no statistically significant difference seen in distribution of these variables.

However, the two groups differed with respect to distribution of sex, (p=0.009). Control group had more number of males as compared to cases. But platelet indices did not differ significantly with gender (p>0.05 for MPV, PDW and platelet counts) so this difference in male and female distribution did not affect our results with respect to platelet indices. So, gender is a confounding factor in present study. In present study cases were divided into culture positive (69), only sepsis screen positive (129) and both negative (41). In present study sepsis screen was positive in 129(65.81%) case a while it was negative in 67 (34.18%) of cases (Table 1). Blood culture was positive in 69(35.20%) cases while it was negative in 127 (64.8%) of cases and gram-negative organism were detected in 48 (24.48%), gram positive

21(10.71%) in case group (n=196).out of this most common was *Klebsiella* which was present in 27(39.13%) case.

Table 1: Distribution of cases according to various laboratory parameters for sepsis group (n=196).

Parameter	Positive	Negative
CRP>10mg/L	115(58.67%)	81(41.33%)
Micro ESR>15mm	47(23.97%)	149(76.03%)
TLC<5000 or>25000	70(35.71%)	126(64.29%)
Neutrophils<1800/mm ³	18(9.2%)	188(95.92%)

Out of them 59(85.50%) culture positive cases, 108(83.72%) sepsis screen positive cases and 30(73.17%) both negative cases were having platelet count <1.5 lac/mm³. While 41(20.90%) controls had platelet count <1.5 lac/cmm. In present study 55 (79.71%) culture positive cases, 97 (75.19%) sepsis screen positive cases and 24 (58.53%) both negative cases were having MPV >10.8 fl, 52(26.53%) controls had MPV >10.8 fl. Out of them 49 (71%) culture positive cases, 86 (66.66%) sepsis screen positive cases and 24(58.53%) both negative cases were having PDW >19.1fl, 52(26.53%) controls had PDW >19.1fl (Table 2).

Table 2: Association between various platelet indices and cases and control group.

	Case (culture positive) (N=69)	Case 2 (Only sepsis screen positive) (N=129)	Case 3 (both culture and screen negative (N=41)	Control (healthy babies) (N=196)
Platelet count <1.5 Lac.	59 (85.50%)	108 (83.72%)	30 (73.17%)	41 (20.91%)
MPV >10.8fl	55 (79.71%)	97 (75.19%)	24 (58.53%)	52 (26.53%)
PDW >19.1fl	49 (71%)	86 (66.66%)	24 (58.5%)	68 (34.69%)

Table 3 shows the distribution of neonates based on cut off values of platelet indices in both case and control groups.

Table 3: Association between platelet indices in both case and control groups.

	Study group		
	Cases (196)	Controls (196)	p value
	N (%)	N (%)	
Platelet count			
< 1.5 Lacs/mm ³	159 (81.12)	41 (20.91)	< 0.0001
$\geq 1.5 \text{ Lacs/mm}^3$	37 (18.87)	155 (79.08)	
MPV			
> 10.8 fl	139 (70.91)	52 (26.53)	<0.0001
≤ 10.8 fl	57 (29.08)	144 (73.46)	
PDW			
> 19.1 fl	129 (65.81)	68 (34.69)	< 0.0001
≤ 19.1 fl	67 (34.18)	128 (65.30)	

In present study, 81.12% neonates in case group had platelet count less than 1.5lacs/mm³ while in control group 20.91% neonates only had the same. This difference was statistically significant, (p<0.0001). Similarly, 70.91% neonates in case group had MPV more than >10.8 fl whereas in control group only 26.53% neonates had the same, with difference was statistically significant, (p<0.0001) Similarly, 65.81% neonates in case group and 34.69% in control group had PDW more than 19.1fl and this difference was statistically significant, (p=0.0001). Table 4 shows difference of mean values of platelet count, MPV and PDW between case and control groups is depicted in table 4. Here we

observed that the mean platelet count was 1.07 \pm 0.698 lacs/mm³ in cases and 2.04 \pm 0.759 lacs/mm³was in controls group, being significantly less in case group than in control group, (p<0.0001). The mean MPV value was 11.82 \pm 1.69 fl in cases and 9.75 \pm 1.45 fl was in controls, significantly more in case group than in control group, (p<0.0001). The mean PDW value was 20.62 \pm 2.22 fl in cases and 18.64 \pm 1.96 fl was in controls, significantly more in case group than in control group, (p<0.0001).

Table 4: Difference of mean values of platelet indices between case and control groups.

	Study group		n volue
	Cases	Controls	p value
	Mean±SD	Mean±SD	
Platelet count (Lacs/mm ³⁾	1.07±0.698	2.04±0.759	< 0.0001
MPV (fl)	11.82±1.69	9.75±1.45	< 0.0001
PDW (fl)	20.62±2.22	18.64±1.96	< 0.0001

Study shows the difference of proportion of neonates based on cut offs values of various platelet indices between pre-term and term babies in case group. In present study group 84(81.55%) preterm and 75(80.64%) term neonates were having a platelet count <1.5 lacs/mm3and the difference was not significant, (p<0.871).

Similarly, 71(68.93%) preterm and 68(73.11%) term neonates were having MPV >10.8fl with difference being insignificant, (p<0.626). In present study group 66(64.07%) preterm and 63 (67.74%) term neonates had PDW >19.1fl with difference being not significant, (p=0.697)

When we correlate the cut off values of various platelet indices with age groups in cases. In present study 95(87.38%) neonates of more than 72 hours age group and 64(73.56%) neonates with age less than 72 hours had a platelet count <1.5 lacs/mm3with difference being statistically significant, (p=0.02).

Similarly, 86(78.64%) neonates with age more than 72 hours and 53(60.64%) neonates less than 72 hours age group were having MPV >10.8fl, with difference was statistically significant, (p=0.009), Again, 77(72.82%) neonates with age more than 72 hours and 52(64.36%) neonates less than 72 hours age group were having PDW >19.1fl, with difference was statistically significant, (p=0.0149).

When we compared gm +ve and gm -ve sepsis, no significant differences in MPV, PDW and platelet counts were observed, (P >0.05 for all parameters)

It was seen that a platelet count <1.5lacs/mm3 was most sensitive marker for sepsis, sensitivity 83.70% followed by MPV (75.20%) and PDW (66.70%). The specificity of platelet count <1.5 lacs/mm3 was also the highest (65%) followed by MPV (64.30%) and PDW (57.80%)

When any two of the platelet indices were combined the specificity increased to maximum 67.0% in platelet count and MPV combined group. The sensitivity was being maximum (85.80%) with a combination of platelet count and MPV as marker for sepsis. However, when all three were taken together the sensitivity of the test was 84.10% and specificity was (65.50%).

DISCUSSION

Neonatal sepsis is associate with high mortality as diagnosis of sepsis in neonates' presents as a challenge because the clinical signs of sepsis are non-specific, and it mainly depended upon investigation. Among these blood cultures is the gold standard for the diagnosis for neonatal sepsis but it's utility is limited due to delayed reporting and low positivity. To overcome this limitation and to guide early diagnosis of neonatal sepsis, sepsis screens (CRP, micro ESR, hematological parameters) are used. The negative predictive value of various sepsis screen parameters is too low to confidently rule out early onset sepsis. In addition, their use will increase the cost of investigations significantly

There have been studies showing the significant changes in platelet indices in patients with neonatal sepsis. These studies measure Platelet count, MPV (mean platelet volume) and platelet distribution width (PDW). These parameters may increase the sensitivity and specificity of the existing sepsis screen when combined with it. It has been seen that platelet count decreases and mean platelet volume (MPV) and platelet distribution width (PDW) increase in neonates with sepsis, high MPV and PDW show high specificity for detecting bacteremia (95% and

79% respectively) and have good negative predictive value.⁴ In the present study, analysis of demographic variables (birth weight, gestational age) showed that these variables did not differ between cases and controls except for the gender, in which control had more number of males compared to case, (p =0.01). But platelet indices did not differ significantly with gender so this difference in male female distribution did not affect our results with respect to platelet indices.

In present study the two groups were significantly different when mean of platelet indices were compared. The platelet count was lower than 1.5 lacs/mm³ in neonatal sepsis group, (p<0.0001). The MPV was increased above 10.8 fl (p<0.0001) and PDW was increased above 19.1fl (p<0.0001) in the cases as compared to controls. In early onset (<72 hours.) and late onset (>72 hours.) sepsis, platelet counts, MPV and PDW have significant difference, (p<0.05). It was seen that decrease platelet count, increase MPV and increase PDW frequently seen in late onset sepsis group, (p=0.02, p=0.009 and p=0.0149 respectively).

Of the preterm babies 52.55% had features of neonatal sepsis while 47.44% of the term babies developed neonatal sepsis. This figure was slightly higher in preterm babies in present study. This difference of sepsis in preterm and term in was seen because preterm babies are immunologically immature and are more prone for sepsis. When preterm and term babies with sepsis were compared, there are no significant differences in MPV, PDW and platelet counts (p>0.05) suggesting that platelet indices do not vary with gestational age.

When we combined sepsis screen and platelet indices, the sensitivity was decreased, and the specificity increased. Highest sensitivity and specificity was seen with combination of sepsis screen and (platelet counts + MPV + PDW), which was 90.60%, 78.90% respectively.

Sepsis screen and its relation with blood culture

Out of 196 enrolled newborns in present study CRP was positive in 115 (58.67%), abnormal total leukocyte count in 70 (35.71%), absolute neutrophil count less than 1800cells/per cubic mm in 18 (9.2%) and micro-ESR was in 47 (23.97%) cases. Perinatal sepsis score was positive in 37 (18.87%) cases and in 04 (2.04%) control group. Blood culture was positive in 69(35.20%) cases out of a total of 196. Our observations are comparable with the result of Okascharoen et al who stated that blood culture positivity in neonatal sepsis is only 20-30%.²

In present study out 69 culture positive cases, of the organisms isolated, 21(30.43%) were gram positive organisms and 48 (69.56%) were gram negative organisms highlighting the fact that gram negative sepsis is more prevalent than gram positive in present study population. This is also comparable with the results of Moreno et al which also shows similar type of results.⁵

Platelet indices in neonatal sepsis

Present study results show that none of the platelet indices varies significantly with respect to sex, gestation age. Abdulla et al have also shown that there is no statistical significance difference in platelets indices with these parameters.⁶

Platelet count

In present study group 81.12% of cases of neonatal sepsis developed thrombocytopenia. The prevalence of thrombocytopenia is variable and different values have been reported by workers across the globe. Studies by Sartaj A. Bhat et al were revealed that 66.25% developed thrombocytopenia in cases of neonatal sepsis.⁷ Ahmed et al also showed that mortality rate was also higher among children with thrombocytopenia and its prevalence was 24.7% in neonatal sepsis.8 This variation may be attributed to the fact that in the present study we considered a cut off of platelet counts less than 1.5 lacs/mm3 and other contemporary studies considered a cut off of 1 lacs/mm³. Present study shows that thrombocytopenia was found more in late onset sepsis than early onset, (p=0.02). Kudawla M also observed that decrease in platelet count was seen more in late onset sepsis group. However, it was 33% and 67% in early and late onset sepsis cases respectively. In present study the sensitivity and specificity of platelet count in detecting neonatal sepsis was found to be 83.70 % and 65.0% respectively in case control groups(n=392) while it was 85.50 % and 21.30% respectively in culture proved group (69) (n=196). As compared to other where sensitivity and specificity was 65.5% and 72% respectively, however both the studies reinforce that thrombocytopenia is quite a specific marker for sepsis. Again, the difference may be attributed to different cut off in different studies.

Mean platelet volume (MPV)

MPV in cases and controls differed significantly between the two groups (P<0.0001). This shows that an increase in MPV is found more frequently in cases than in controls. MPV was found to be increased in 70.7 % of cases in neonatal sepsis which was far greater when compared with the results of study by Abdulla et al which shows 27.8% increases in MPV in case of sepsis.⁶

Increased MPV in early and late onset sepsis are 60.64%.and 78.64% respectively. This difference is significant (p = 0.009). This conclude that increased MPV is seen in late onset sepsis than early onset. Many other studies for example Patrick RH et al have also shown that MPV was increased more in cases with late onset sepsis.⁴ In present study the sensitivity and specificity of MPV(>10.8fl) in detecting neonatal sepsis was found to be 75.20% and 64.30% respectively in case control groups (n=392) while to be 79.70% and 33.90% respectively in culture proved group (69) (n=196). A study by Arad ID et al it was seen that sensitivity and

specificity of increase in MPV in neonatal sepsis is 54% and 46% respectively. 10

Platelet distribution width (PDW)

Present study shows that in cases of sepsis PDW was increased significantly as compared to control group. PDW was increased in 65.81 % of cases in neonatal sepsis which was far greater when compared with the results of other study which shows that PDW increased in 38% cases of sepsis.

When comparing early and late onset sepsis, PDW was increased in 64.36% cases of EOS and 72.82 % cases of LOS. Increase in PDW in early and late neonatal sepsis has a difference significantly between the two groups (p = 0.0149). Patrick RH et al have shown that increased PDW is more commonly seen in late onset sepsis.⁴

Platelet and their indices

In present study we compare the sensitivity, specificity of platelet indices with gold standard blood culture in the case control group. Thrombocytopenia had the highest sensitivity to detect sepsis (85.50%). Abdulla et al were demonstrated that thrombocytopenia as an important marker and they had compared gram positive and gramnegative organism. However, they did not compare blood culture proven sepsis with negative blood culture.6 MPV and PDW had a sensitivity of 79.70% and 71.0% respectively in culture proved in case control group. When we combined all three indices the sensitivity of the study was increase (91.50%) and specificity also increase (65.14%) in (platelet count + MPV + PDW) group. There is no study to the best of our knowledge in who compared these indices to calculate sensitivity and specificity with respect to blood culture as gold standard.

When we compared the existing sepsis screen with blood culture, the sensitivity and specificity was 63.30% and 73.37% respectively. Previous studies have shown the sepsis screen to variable sensitivity and specificity in different studies because the timing of sample collection is important. If sample is drawn several hours after illness or after starting antibiotics sensitivity of sepsis screen will change. CRP increases in other inflammatory conditions. Khassawneh M et al reported that elevated CRP and thrombocytopenia were seen in 28% and 24% of early onset sepsis respectively and in 79.6% and 59.3% of late onset sepsis. 11 According to Philip et al CRP has a sensitivity of 47% for neonatal sepsis.³ In this study CRP levels were raised in 32.3% of neonates with sepsis and 58.5% of neonates with probable sepsis and 9.2% of neonates who were not infected showed an elevated CRP. Maucha et al have reported elevated CRP in 76% cases of neonatal sepsis.12

When we combine the existing sepsis screen and platelet indices and correlate it with blood culture in case control group, the sensitivity and specificity (88.40%), (70.50%)

respectively, in (sepsis screen + platelet count) group compare to platelet indices combine group. However, it must be noted here that the sensitivity of platelet indices alone for identifying sepsis was higher than the existing sepsis screen (62.30%). A combination of platelet indices with sepsis screen further increase their specificity. The specificity of platelet count was high (65.0%) in identifying non-septic babies but it was low (56.96) in identifying babies with a negative blood culture. The specificity of existing sepsis screen itself was also low (73.23%).

Highest specificity was seen with a combination of sepsis screen with (MPV + PDW + Platelet count) 78.90% in culture proved sepsis. Which was high compare to platelet count specificity (56.96%) in culture proved case control group.

CONCLUSION

This study was conducted to establish the relationship between neonatal sepsis and platelet indices. High PDW, high MPV and low platelet count are more associated with neonatal sepsis. So, this study is useful for evaluating platelet indices as a marker of neonatal sepsis alone or with combination to pre-existing sepsis screen. Platelet indices alone (91.50%) had a better sensitivity in identification of sepsis than sepsis screen (62.30%). In combination with sepsis screen their specificity also increases.

Thus, it is concluded that platelet and its indices may be used as a sensitive marker to identify septic babies and it may be combined with existing sepsis screen to specifically exclude non-septic case.

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Institutional Ethics Committee

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